OWD Docket No. 14598-41 (Was: BEH-7443)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	**K. Kasper et al.**	**Examiner:**	**M.E. Ceperley**
Serial No.:	**09/368,010**	**Group Art Unit:**	**1641**
Filed:	**August 3, 1999**	**Docket:**	**14598-41**
Due Date:	**August 7, 2001**		

Title: MONOCLONAL ANTIBODIES TO TACROLIMUS AND IMMUNOASSAY METHODS FOR TACROLIMUS

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service, as first class mail, with sufficient postage, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on August 7, 2001.

By: Joy Johnson
Name: Joy Johnson

DECLARATION OF DR. KENNETH C. KASPER UNDER 37 C.F.R. § 1.132

Honorable Assistant Commissioner for Patents
Washington, DC 20231

Gentlemen:

With respect to the above-identified patent application, I declare as follows:

1. I am one of the inventors of the above-identified patent application. I was an employee of the Syva division of Dade Behring at the time of the filing of the above-identified patent application. I have personal knowledge of the facts recited in this application and could testify competently thereto.

2. As an inventor, I have reviewed the specification and claims of the above-identified patent application and understand the specification and claims.

3. I am one of the authors of the abstract submitted to the 6th International Congress of TDM-CT entitled "A New Monoclonal Antibody for the Immunosuppressive Drug Tacrolimus" ("TDM-CT Abstract"). I am familiar with the contents of the TDM-CT Abstract. A true and correct copy of the TDM-CT Abstract is attached hereto as Exhibit A.

4. The TDM-CT Abstract was published no more than one year before the filing date of the above-identified application.

5. The authors of the TDM-CT Abstract are H.J. Jeong, H. Liu, P. Miller, S.H. Roth, A.T. Brady, G. Bolle, and myself. Of these, all but S.H. Roth, A.T. Brady, and G. Bolle are named inventors of the above-identified patent application.

6. The content of the TDM-CT Abstract derived from work by myself and the other named inventors of the above-identified patent application. The contributions of the persons not named inventors who were authors of the TDM-CT Abstract, namely, S.H. Roth, A.T. Brady, and G. Bolle, were made at the direction and supervision of one or more of the named inventors of the above-identified patent application. This direction and supervision is a common and customary part of team research and development as carried out at the Syva division of Dade Behring.

7. S.H. Roth was not responsible for the development of the antibody. However, he was responsible for data contributed to the TDM-CT Abstract in using the antibody for the development of an immunoassay. As such, he was properly named an author on the TDM-CT Abstract but was not properly named an inventor on the above-identified patent application.

8. Similarly, A.T. Brady was not responsible for the development of the antibody. However, she was also responsible for data contributed to the TDM-CT Abstract in using the antibody for the development of an immunoassay. As such, she was properly named an author

on the TDM-CT Abstract but was not properly named an inventor on the above-identified patent application.

9. Along the same lines, G. Bolle was not responsible for the development of the antibody. However, he also was responsible for data contributed to the TDM-CT Abstract in using the antibody for the development of an immunoassay. As such, he was also properly named an author on the TDM-CT Abstract but was not properly named an inventor on the above-identified patent application.

10. These three individuals, S.H. Roth, A.T. Brady, and G. Bolle, named as co-authors of the TDM-CT Abstract were not inventors of the subject matter of the above-identified patent application, but were listed as co-authors of the TDM-CT Abstract in order for them to receive credit for participation in the research program leading to the work described in the TDM-CT Abstract in view of their contributions as discussed above.

11. Two inventors, Dariush Davalian and Denise L. Williams, were not named as co-authors of the TDM-CT Abstract. These two individuals were not named as co-authors because, even though they contributed to the subject matter of at least one claim of the application, they were not responsible for the data presented in the TDM-CT Abstract. Dariush Davalian was instrumental with respect to the development of the immunogen, and Denise L. Williams was instrumental in isolating the cell line. However, these two individuals did not participate at all with respect to data that described performance of the antibody in a specific assay situation as described in the TDM-CT Abstract.

12. Accordingly, the work described in the TDM-CT Abstract that is part of the subject matter of the above-identified patent application is properly attributable to the inventors of the above-identified patent application.

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I further declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

~~Executed at~~ Victoria, British Columbia, Canada, on August 7, 2001.

Dr. Kenneth C. Kasper

63 **DISTRIBUTION OF TACROLIMUS IN BLOOD.**

RA Nand[1], AJ McLachlan[1], AM Keogh[2], KF Brown[1].
Department of Pharmacy, University of Sydney, Australia[1]; Heart and Lung Transplant Unit, St Vincent's Hospital, Darlinghurst, Australia[2].

Tacrolimus (T, Prograf), a potent immunosuppressive drug used in organ transplantation, exhibits considerable inter- and intra-patient pharmacokinetic variability making dose individualisation difficult. The blood distribution of tacrolimus has been studied *in vitro* previously (1-3), however the reported conclusions in human blood were conflicting. We have characterised the *in vitro* distribution of ^{3}H-tacrolimus (^{3}H-T) in blood of healthy subjects. Blood and plasma samples (n=6) were spiked with ^{3}H-T (2 µCi) after purification by HPLC. After incubation (for 60 min at 37°C), plasma proteins and blood cells were separated by density gradient ultracentrifugation. ^{3}H-T was analysed using radiochemical analysis. Drug distribution in each fraction was expressed as a percent of total dose counts. In blood cells, ^{3}H-T was associated with the erythrocytes (94.2 ± 1.6 %), plasma (5.6 ± 1.7 %) and lymphocytes (0.18 ± 0.07 %). In plasma, ^{3}H-T was associated with the lipoprotein deficient serum (61.2 ± 3.5 %), high density lipoproteins (28.1 ± 5.36 %), low density lipoproteins (7.9 ± 1.6 %), and very low density lipoproteins (1.42 ± 1.38 %). Thus changes in haematocrit and plasma proteins (albumin and lipoproteins) will affect the unbound concentration of tacrolimus. *In vitro* distribution studies of tacrolimus in transplant recipients are needed to elucidate the factors that control its distribution and to enable understanding of the inter- and intra-patient variability.

(1) Rifai N, et al. *Clin Biochem* 1996; 29:149-155.
(2) Takada K, et al. *Biopharm Drug Dispos* 1993, 14:659-672.
(3) Warty V, et al. *Trans Proceedings* 1991, 23:954-955.

64 **EVALUATION OF SPECIFICITY OF MONOCLONAL ANTIBODIES TO SIROLIMUS**

AJ Malcolm[1], MS Hueter[1], M Abel[1], S Naicker[1], RW Yatscoff[1], CJ Haley[1], KC Kasper[1]. Isotechnika Inc, Edmonton, Alberta, Canada[1]; Syva Company (a subsidiary of Dade Behring Inc), San Jose, USA[1].

Sirolimus is a new macrolide immunosuppressive agent that is metabolized via the cytochrome P450 3A enzymes to produce demethylated and hydroxylated metabolites. Fourteen monoclonal antibodies to sirolimus were evaluated for specificity with respect to tacrolimus and 5 sirolimus metabolites: 39-O-demethyl-, 16-O-demethyl-, 27,39-O-didemethyl-, and 2 mono-hydroxylated metabolites (H1, H2). Using an Emit® assay format, each antibody was optimized to generate a standard curve from 0-15 ng/mL sirolimus in a buffer matrix. Tacrolimus and each metabolite were assayed at 5 ng/mL. Percent cross-reactivities were:

Clone	39-O-deM	16-O-deM	27, 39-O-dideM	H1	H2
6	44	59	76	1	0
5	40	69	81	5	5
8	51	73	73	6	4
7	50	57	70	10	11
14	33	28	23	48	0
9	38	28	30	33	0
13	47	51	27	60	1
10	47	51	27	60	1
4	44	53	50	56	3
2	35	50	40	46	3
12	42	37	25	43	8
1	43	47	48	69	15
11	53	43	48	63	17
3	50	51	57	52	18

Tacrolimus was not recognized by any antibody. Cross-reactivity to demethylated metabolites varied. Antibodies could be grouped based on cross-reactivity to hydroxylated metabolites: 4 antibodies did not cross-react with either H1 or H2; 7 antibodies cross-reacted with H1 but not H2; and 3 antibodies cross-reacted with both. The clinical relevance of the metabolite cross-reactivity is not known.

65 **A NEW MONOCLONAL ANTIBODY FOR THE IMMUNOSUPPRESSIVE DRUG TACROLIMUS.**

HJ Jeong, H Liu, P Miller, SH Roth, AT Brady, G Bolle, KC Kasper, Syva Company (a subsidiary of Dade Behring Inc.), San Jose CA, USA.

A high affinity monoclonal antibody for tacrolimus (FK506) with a unique metabolite cross reactivity was produced. A research assay was developed using a homogenous enzyme immunoassay (0 to 30 µg/L) based on the Emit® technology. The assay consisted of a methanolic-CuSO$_4$ sample extraction of FK506 from the blood sample and immunoassay reagents.

The supernatant from the centrifuged sample was decanted into sample cups for testing on the COBAS MIRA® (Roche Diagnostic Systems). Each of the FK506 metabolites (Isotechnika, Inc.), MI, MII, MIII, MV, and MVI, were spiked at 3.0 µg/L into blood hemolysate containing 10.0 µg/L FK506. MI was the only metabolite that cross-reacted; the MI/FK506 sample measured as 12.4 µg/L. Metabolites MIV, MVII to MIX still require testing.

A sample correlation compared 40 transplant patient samples with results from the Abbott IMx® Tacrolimus II Assay: Emit = 0.840 (IMx) + 0.618, R = 0.978. IMx calibrators and controls were assayed with Emit: Emit = 0.830 (IMx) + 0.204, R = 0.999, N = 5. Differences in FK506 calibrator assignment between IMx and the research assay could explain the slope of the method comparison.

The Emit FK506 research assay demonstrated a new antibody for FK506 with cross-reactivity to MI metabolite and acceptable correlation with the IMx assay. This Emit assay has not been tested clinically. Further study with this antibody may help understand the clinical relevance of the metabolite cross-reactivity in patient samples.

66 **DEVELOPMENT OF A FULLY AUTOMATED CSA METHOD FOR THE DIMENSION® CLINICAL CHEMISTRY SYSTEM.**

DM Obzansky[1], LM Shaw[2], C Wang[1], SM Hernandez[1], LB Fields[1], RC Wright[1], JS Masulli[1], B Woods-Galvin[1], RK Wiedenmann[1], JP Swyka[1], SP Kramer[1], A Chatterjee[1], MM Mendola[1].
(Dade Behring Inc., Newark, Delaware[1]; University of Pennsylvania Medical Center, Philadelphia, Pennsylvania[2]).

A fully automated CSA method has been developed for the Dimension® clinical chemistry system. The sample probe first ultrasonically mixes EDTA whole blood and delivers 5 µL into a reaction vessel containing lysing reagent. Excess antibody - beta galactosidase conjugate is added, followed by incubation and addition of CSA-chromium dioxide particles. After separating this reaction mixture, the CSA bound conjugate is transferred to a cuvette and its enzymatic rate measured. Total assay time is less than 12 minutes.

The method is linear to 500 ng/mL and has a sensitivity of less than 25 ng/mL. The calibration curve is stable for 30 days. In a reproducibility experiment using NCCLS protocol EP5, within-run reproducibility (CV) was 3.5%, 3.3%, 3.4%, 2.5%, 2.1% and 1.5% and total reproducibility (CV) was 6.3%, 6.5%, 6.0%, 4.2%, 2.1% and 2.5% at 72, 117, 140, 218, 387 and 447 ng/mL CSA, respectively. Metabolite crossreactivity, evaluated in the presence of 1000 ng/mL metabolite and 200 ng/mL CSA, was: ≤ 0.5% for AM19, AM1, AM1c and AM1c9, 3.5% for AM9, 2.7% for AM4N, and 0.8% for AM4n9. Analysis of whole blood samples from 44 heart, 18 liver, 9 lung and 75 renal transplant patients on the TDx® analyzer (TDx), Dimension® system (DM) and by HPLC gave the following statistics:

X	Y	Slope	Intercept	r	Sy/x
HPLC	DM	1.04	5.7	0.95	40.1
HPLC	TDx	1.43	23.0	0.92	63.1
DM	TDx	1.33	20.4	0.92	64.2

A comparison between the Dimension® system CSA method and the EMIT® CSA method on the Cobas Mira® using blood from 19 bone marrow, 43 heart, 191 kidney, 20 liver and 18 lung patients, gave the following statistics: DM = 1.03 EMIT + 18.5, r = 0.98, Sy/x = 23.6.

Thus, this fully automated CSA method provides accurate and precise results for the measurement of whole blood cyclosporine.

EXHIBIT A